

The University of Chicago

HISTOLOGICAL VARIATIONS IN
COSMOS IN RELATION TO
PHOTOPERIODISM

A DISSERTATION SUBMITTED TO THE
FACULTY OF THE DIVISION OF THE BIO-
LOGICAL SCIENCES IN CANDIDACY FOR
THE DEGREE OF DOCTOR OF PHILOSOPHY

DEPARTMENT OF BOTANY

1934

By
ORLIN BIDDULPH

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HISTOLOGICAL VARIATIONS IN COSMOS IN RELATION TO PHOTOPERIODISM

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY 463

ORLIN BIDDULPH

(WITH TWENTY-NINE FIGURES)

Introduction

GARNER and ALLARD (8, 9, 10) have described the response of *Cosmos* when subjected to various photoperiodic treatments. Their investigations show that *C. sulphureus* Cav. is a short day plant growing vegetatively in long days and flowering when the daily light period is shortened to ten hours. It can be grown to a height of 15 feet or more or made to flower in 50 days, depending on the length of the daily light period. The age and size of the plants make no difference in the speed with which they react to the change in the length of the daily light period. By the use of an electric light to prolong the light period of the normal day, it was found that an intensity of 4-foot candles was effective in producing vegetative growth in short day plants and reproductive activity in long day plants.

When short day plants which have been given a 10-hour day for 15 days are transferred to conditions of a long day, only a few of the blossom buds already initiated finally open, the others being permanently suppressed. Vegetative shoots from the axillary buds on the axis below the apical flower bud soon begin to elongate and the latter is apparently deprived of the necessary nutritive materials for development.

GARNER, ALLARD, and BACON (11) have applied the concept of the carbohydrate-nitrogen relation as a factor in flowering and reproduction (16) to photoperiodic response, and have found that a change in soluble carbohydrate accompanies a change in the length of the daily light period. In *Cosmos* there is an increase in reducing sugars in the apex of the stem within 48 hours after the transfer of the plant from long to short days.

NIGHTINGALE (19) has stated that in general plants high in carbohydrates have relatively much of their assimilated nitrogen as protein, although the percentage of protein may be low, and has suggested that the duration of the light period is a factor in the utilization of carbohydrate for the conversion of inorganic nitrogen into protein form. A shift in the day length brings about a reworking of the metabolized products and an establishment of a new equilibrium between soluble and insoluble forms. Vegetative activity prevails when a high percentage of the metabolized nitrogen is present in a relatively soluble form, whereas flowering and reproduction are accompanied by an accumulation of carbohydrate and a decrease in soluble forms of nitrogen.

ADAMS (1, 2) has shown that plants exposed to a diminished light supply have a more rapid growth rate at first but ultimately the ones receiving the most light make the most growth. GILBERT (12, 13), in his work on the photoperiodic responses of *Xanthium*, found that under both high and low temperature conditions the ratios of total carbohydrate to total nitrogen and soluble carbohydrate to soluble nitrogen were distinctly ascending as flower primordia were formed.

ECKERSON (6) has found in Biloxi soy beans grown in an 8-hour day and a 16-hour night a decrease in nitrate reducing power of more than 95 per cent, and KNOTT (15) has found that the proper day length for the induction of flowering in *Cosmos* reduced the catalase activity in the stem tip to an extent of 109 per cent in 20 days. The length of the daily light period may also control the relative amounts of carotenoid pigments (18). CHIBNALL (5) found that at the time of flower bud formation asparagine is the most prominent product of the protein metabolism.

These investigations indicate that there are definite physiological changes accompanying flowering and reproduction. These changes have been induced entirely by the length of the daily light period as the external nutrient supply has remained constant. It is possible then to induce flowering entirely apart from any nutrient treatment. Flowering so induced is accompanied by a definite chemical change in the region of the apical primordium. The present study repre-

sents an attempt to determine more precisely the exact anatomical response of the primordium to the day length and the progressive chemical changes accompanying or preceding such anatomical change.

Methods

Plants of *Cosmos sulphureus* Cav. var. Klondike were grown from seed in rich soil, in boxes $1 \times 1 \times 1$ feet. They were kept under long day conditions by the use of 1000 watt Mazda lamps supplying an intensity of approximately 75 foot candles at the tops of the plants. Plants were grown under both 15- and 16-hour days, the lamps supplementing the particular number of daylight hours prevailing at the time of the experiment. Ordinary greenhouse temperatures prevailed. During the short day treatment the plants were kept at the same temperature as the long day controls. In the series run in May the night temperature varied between 65° and 70° F. while the day temperature rose to 90° or 100° F. for a short period during the afternoon. In the series grown in January the temperature remained nearly constant at 76° during the day and 65° F. at night.

Two methods of artificially producing short days were used. In May, when the temperatures were high at night, a screen of double thickness black sateen cloth was placed over the framework surrounding the plants at 4:30 P.M. and removed at 8:30 A.M., giving an 8-hour day. In winter the boxes were placed on a truck and moved into a dark chamber at 4:30 P.M. and out at 9:00 A.M., giving a $7\frac{1}{2}$ -hour day.

Only relatively short periods of short day treatment are necessary to induce flowering in *Cosmos*. Accordingly separate groups of plants were given 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, and 14 consecutive short days respectively, and then exposed to long day treatment. Plants of each group were then examined periodically to determine the least number of short days necessary to induce flowering. The retarding effect of the subsequent long day treatment was determined by comparison with plants under short day treatment. A dissecting microscope proved the most useful means for determining the morphological response of the apical primordium. Material was also imbedded and prepared according to the usual paraffin method.

Microchemical as well as macrochemical analyses were made at various stages indicated as being significant by the detailed anatomical examination.

Investigation

MICROCHEMICAL METHODS

Periodic examinations were made in order to follow the progress of the chemical changes occurring after the short day treatment was begun. Apical primordia of primary or lateral axes of purely vegetative long day plants were used in each case as a standard of comparison. The primordium itself and only the very closely associated stem tissue were used in the stem tip sample. The base of the stem was also examined to determine whether or not there was a change in this region accompanying the changes in the primordium. Comparable sections through stem tips of the long and short day plants were made by means of a sliding microtome in which both stems had been fastened by clamping them side by side in cotton, so that both were sectioned by the same stroke of the knife.

The microchemical tests found to be the most useful were:

GLUTATHIONE.—Based on the nitroprusside reaction of HOPKINS, modified by FINK (7), this was used as follows: Heat sections in 10 per cent acetic acid to boiling and remove to three drops of saturated ammonium sulphate. Add two drops of 5 per cent sodium nitroprusside ($\text{Na}_2\text{Fe}(\text{CN})_5 \cdot \text{NO} \cdot 2\text{H}_2\text{O}$). Allow to stand for 5 minutes. Add an excess of concentrated ammonium hydroxide (two or three drops). A pink to permanganate color indicates the presence of glutathione. The depth and duration of the color have been shown to indicate the amount present.

ARGININE.—Based on the SAKAGUCHI (21) reaction which is specific for the free-guanidine group and therefore for arginine, this was used as follows: Place sections in one drop of 15 per cent NaOH. Add two drops of 0.15 per cent alpha naphthol. Add three or more drops of 0.5 per cent sodium hypochlorite. Red color on standing indicates arginine. It may be used quantitatively.

HISTIDINE AND TYROSIN.—Based on Ehrlich's diazo reaction, this was used as follows: One drop of 0.5 per cent solution of sulphanilic acid in 2 per cent hydrochloric acid. Allow to react with one drop

of 0.5 per cent sodium nitrite for a minute or two. Add section to this and make the mixture alkaline with 10 per cent sodium carbonate. The presence of histidine is indicated by a deep red color. Tyrosin gives more of an orange tinge. The reaction can be employed quantitatively.

CONDENSATION REACTIONS WITH TRYPTOPHAN.—Treat sections with a 5 per cent solution of formalin for 15 minutes, then add strong hydrochloric acid to the mixture. A trace of an oxidizing agent such as ferric salts or nitrites speeds the reaction.

TYROSIN.—Based on the use of Millon's reagent as modified by BENSLEY (3).

TOTAL PROTEIN.—Based on various reactions; with I_2KI , ninhydrine, Millon's reagent, Sakaguchi reaction, etc.

FRUCTOSE.—Based on the reduction of Fehling's solution in the cold.

TOTAL REDUCING SUGAR.—Based on the reduction of Fehling's solution when heated.

Inulin, which is a storage product of *Cosmos*, seems to have a peculiar effect on the reduction of Fehling's solution by the reducing sugars, causing the formation of an amorphous copper complex which contains some crystals of Cu_2O in its matrix. This difficulty was minimized by diluting the Fehling's solution (one part of mixed Fehling's solution to three parts of water). Crystals of Cu_2O are also larger if this concentration is used.

MACROCHEMICAL ANALYSES

After the critical number of short days necessary to induce flowering was determined and the subsequent response of the primordia known, it was possible to bring the plants to any desired anatomical stage for macrochemical analysis. A period of ten short days was selected as an appropriate treatment of material to show the chemical differences between plants which had received the necessary stimulus to flower and those which would remain strictly vegetative under long day conditions.

The average height of the plants used for analyses was 12 inches. The stems were cut just above the ground and divided into two parts, the stem tip sample including the first inch of stem tip, and

the stem base sample including the remainder of the stem. Nitrogen fractionations and carbohydrate determinations were made on each sample.

ANATOMICAL OBSERVATIONS

All plants do not react to short day treatment with exactly the same speed. Those which react the fastest may precede the slower



FIGS. 1-9.—Figs. 1-3, median sections through primordium of plants which have received seven 8-hour days followed by seven 16-hour days; figs. 4-6, seven 8-hour days followed by fourteen 16-hour days; figs. 7-9, seven 8-hour days followed by eighteen 16-hour days.

plants in the formation of flower buds by two or three days. Seven short days was the least number which produced an anatomical response, regardless of whether a $7\frac{1}{2}$ -hour or an 8-hour daily light period was used. The plants which were exposed to an 8-hour daily light period in May responded 100 per cent within 9 days, whereas ten days were required for 100 per cent response under a $7\frac{1}{2}$ -hour

daily light period in January. Figures 1-9 show the variations in the response of *Cosmos* primordia to seven 8-hour days in May.

It was thought that by reducing the length of the period of short day treatment to the absolute minimum necessary for the differentiation of flower primordia and then exposing the plants to the conditions of the long day, some expression of interphases (or "vegetative



FIGS. 10-18.—Fig. 10, view of primordium of a plant which has changed from an opposite to a spiral leaf arrangement; fig. 11, apical primordium from a plant which has received fourteen $7\frac{1}{2}$ -hour days; fig. 12, twenty-one $7\frac{1}{2}$ -hour days (the two sets of bracts are much further developed than the individual flower primordia); fig. 13, nine $7\frac{1}{2}$ -hour days (no bracts formed at this time); fig. 14, nine $7\frac{1}{2}$ -hour days followed by seven 15-hour days (first row of bracts just developing); fig. 15, nine $7\frac{1}{2}$ -hour days followed by fourteen 15-hour days; fig. 16, eight $7\frac{1}{2}$ -hour days; fig. 17, eight $7\frac{1}{2}$ -hour days followed by seven 15-hour days; fig. 18, eight $7\frac{1}{2}$ -hour days followed by fourteen 15-hour days.

flowers") would appear. In the group of plants given short day treatment in May, however, little tendency toward interphases appeared, save for the change from opposite to spiral leaf arrangement in 18 per cent of the plants (fig. 10).

The fact that all plants in the process of differentiating flower primordia gave strong evidence, after three weeks of subsequent long day treatment, of developing normal flowers, indicated that the light intensity or the daily light period might be further reduced and

a still closer approach to the minimum light requirement for flowering found. The same type of experiment was therefore made in January when the light intensity in Chicago is much lower. The daily light period was reduced to $7\frac{1}{2}$ hours. Groups of plants were exposed to 6, 7, 8, 9, and 10 short days respectively, after which they

TABLE I

RESPONSE OF COSMOS PLANTS TO DAILY LIGHT PERIOD OF 8 HOURS
IN MAY, EXPRESSED IN PERCENTAGE OF NUMBER
OF PLANTS USED

NO. OF SHORT DAYS	REMAINING VEGETATIVE	CHANGING TO SPIRAL	FORMING FLOWER PRIMORDIA	NO. OF PLANTS
6.....	100			9
7.....	41	18	41	39
9.....			100	6

TABLE II

RESPONSE OF COSMOS PLANTS TO DAILY LIGHT PERIOD OF $7\frac{1}{2}$ HOURS
IN JANUARY, EXPRESSED IN PERCENTAGE OF
NUMBER OF PLANTS USED

NO. OF SHORT DAYS	REMAINING VEGETATIVE	CHANGING TO SPIRAL	FORMING FLOWER PRIMORDIA	NO. OF PLANTS
6.....	100			10
7.....	67	20	13	15
8.....	40	11	49	26
9.....	20	27	53	15
10.....			100	10

were removed to conditions of the long day. Electric lights (1000 watt Mazda lamps, giving approximately 75 foot-candles intensity at the top of the plants) were used to supplement the normal day length, giving a 15-hour day. The results obtained are shown in tables I and II.

The differences in the rates of development of flowers by plants which had received 7, 8, and 9 short days are shown in figures 10-18. The retarding effect on floral development produced by long days following the short day treatment may be seen by comparing plants

that were shifted to long day conditions with those that were maintained continuously under short day illumination (figs. 11, 12).

A third group of plants which had attained a height of 12-16 inches was divided into three lots. The first lot received eight $7\frac{1}{2}$ -hour days, the second, nine $7\frac{1}{2}$ -hour days, and the third, ten $7\frac{1}{2}$ -hour days, each being immediately placed under conditions of the long day after the treatment. These plants were allowed to grow in the long day conditions (15 hours of light per day) for three months. At the end of this period a very decided difference in the floral development was found.

The normal *Cosmos* flower has two rows of involuclral bracts, usually eight in each row. The outer set is foliaceous and spreading while the inner set is broader and nearly membranous. The ray flowers, usually eight in number, appear in the axils of the inner set of involuclral bracts. Each disk flower is borne in the axil of a bractlet on the determinate composite head. The bracts ordinarily remain shorter than the disk flowers and are membranous in nature.

The first flowers to open in the foregoing group were those which had received ten short days. Two months of long days were required before the first flowers opened. The control plants, which were kept under continuous short day conditions, began to blossom in six weeks. The following differences in the flowers were noticed (figs. 26-29):

Continuous short day treatment until flowering ($7\frac{1}{2}$ -hour days)

Length of first row of bracts..... 5 mm. (fig. 28)

Length of second row of bracts..... 9 mm.

No. of ray flowers...8; length..... 20 mm. (fig. 27)

Ten short days followed by two months of long days ($7\frac{1}{2}$ - and 15-hour days)

Length of first row of bracts..... 16 mm. (fig. 29)

Length of second row of bracts..... 12 mm.

No. of ray flowers...14; length..... 20 mm. (fig. 26)

Figures 19 to 25 show the types of variations from normal flowers which were found. There are four important points concerned with their development. First, the primordium initiates a flower and becomes determinate, but owing to the influence of the long days the bracts and bractlets elongate and become foliaceous and the flowers



FIGS. 19-29.—Figs. 19, 20, apex of stems from plants which have received eight $7\frac{1}{2}$ -hour days followed by sixty 15-hour days (bracts have become foliaceous and flowers are suppressed); fig. 21, eight $7\frac{1}{2}$ -hour days followed by sixty 15-hour days (flower bud suppressed); figs. 22-24, developing flowers from plants which have received nine $7\frac{1}{2}$ -hour days followed by sixty-five 15-hour days (division of flower primordium shown); fig. 25, nine $7\frac{1}{2}$ -hour days followed by sixty-five 15-hour days (abnormal elongation of stem between bracts and flowers shown); figs. 26, 29, front and rear views of flower from a plant which has received ten $7\frac{1}{2}$ -hour days followed by sixty 15-hour days; figs. 27, 28, front and rear views of flower from a plant which has received continuous treatment under $7\frac{1}{2}$ -hour daily light period.

fail to develop beyond mere appendages in their axils (figs. 19, 20). Second, if the tendency to flower is a little stronger the flower head may develop to a diameter of about 3 mm. and then become permanently suppressed. The axillary buds immediately below become active vegetatively and develop into new shoots which assume apical dominance (fig. 21). Third, if the tendency to flower is still stronger the flower head may develop and produce a mature flower but there is a marked tendency for the primordium to divide, resulting in two flower heads. The division may result in two flower stalks of equal length, or the division may not result in two flower stalks but in the appearance of two flower heads on one stalk (figs. 23, 24). Fourth, when there is considerable retardation of floral development by the long days, an internode of considerable length may develop between the involucre bracts and the bractlets of the floral head (fig. 25); or the elongation of the stem in the region of the involucre bracts may result in their separation longitudinally, leaving them spirally arranged over a distance of 1-2 cm. of the stem immediately below the flower head.

MICROCHEMICAL RESULTS

Vigorously vegetative plants were taken from a 15-hour day and given a $7\frac{1}{2}$ -hour day. Thereafter examinations were made daily at 9:00 A.M. and 7:00 P.M. for a period of 14 days. Long day strictly vegetative plants were used as a standard of comparison at each examination.

From the morning and evening examination of the primordia a diurnal fluctuation in the glutathione content was discovered. It was found that in the morning the primordia of the short day plants contained a considerably greater quantity of glutathione than those of the plants which had received a long day. After both plants had been exposed to a period of light lasting until evening, however, the glutathione content of the primordia was approximately the same. This nightly fluctuation of glutathione in the short day plants continued until about the seventh day, when it became and remained permanently greater than in the vegetative plants. This observation was extended to the fourteenth day, at which time the flower primordium is well developed.

CARBOHYDRATES.—A marked increase in reducing sugars appeared in the stem tips of the short day plants by the fourth day of treatment. From that time on to the fourteenth day, when observations were discontinued, there was a progressive increase in the amount present. The accumulation of reducing sugar appeared before there was any marked increase in the protein content of the stem tip.

NITROGENOUS FRACTIONS.—There was no marked increase in the total amount of organic nitrogenous material in the stem tips of the short day plants during the fourteen days of observation. The relative amount of soluble and insoluble organic nitrogenous products, however, changed markedly. In the stem tips of the plants which had received short day treatment there was an accumulation of proteinaceous material beginning about the fifth or sixth day and becoming marked by the seventh day, from which time it continued to increase. The protoplasm of the cells increased in density.

As the reactions employed for the detection of amino acids are given by both the free amino acids and proteins, there was some difficulty in the determination of the relative amounts of protein and soluble organic nitrogen. This difficulty was best overcome by denaturing the proteins before the color reactions were employed. The proteinaceous material then gave a much better test with Millon's reagent and ninhydrine reactions than undenatured material. With reasonable care in the preparation of the sections, and the comparison of one section with another, there was little difficulty in demonstrating the greater accumulation of complex protein material in the stem tips of the short day plants than in the vegetative tips of long day plants.

MACROCHEMICAL RESULTS

The tenth day after the beginning of the short day treatment was selected as the best time for the collection of samples for analysis, for by that time all plants would have received the necessary stimulus to flower yet there would have been no marked anatomical change. Purely vegetative long day plants were used as the controls.

Carbohydrate analyses were made by mincing the fresh tissue and extracting with hot 95 per cent alcohol. The soluble reducing sugars were determined therefrom by the Bertrand volumetric method.

The insoluble residue from the extraction was boiled in 2.5 per cent hydrochloric acid for three hours and the hydrolyzable carbohydrates determined by the same method. Sucrose was not determined.

Fresh tissue only was used for nitrogenous fractionations. Extractions were made with water according to the methods of CHIB-

TABLE III

ANALYSIS OF PLANTS HAVING RECEIVED 10 SHORT DAYS, AND LONG DAY
VEGETATIVE PLANTS, EXPRESSED IN PERCENTAGE OF GREEN MATTER

	SHORT DAY STEM TIPS	LONG DAY STEM TIPS	PER- CENT- AGE DIF- FER- ENCE	SHORT DAY STEM BASES	LONG DAY STEM BASES	PER- CENT- AGE DIF- FER- ENCE	SHORT DAY WHOLE STEM	LONG DAY WHOLE STEM	PER- CENT- AGE DIF- FER- ENCE	PER- CENT- AGE VARIA- TION OF SAMPLES
NH ₃	0.0032	0.0128	75.0	0.0047	0.0095	50.5	0.0079	.0223	64.6	15
Amide.....	0.0378	.0284	24.7	.0140	0.0182	23.1	.0518	.0466	10.0	15
NO ₃	.0061	.0061	00.0	.0054	.0090	40.0	.0115	.0151	23.8	15
Soluble organ- ic N.....	.1247	.1328	6.1	.1497	.1366	8.7	0.2726	.2694	1.2	2
Protein N....	.2315	.2155	6.9	.0820	.1080	24.1	.3125	.3235	3.4	2
Total organic N.....	.3562	.3483	2.2	.2299	0.2446	6.0	.5861	.5929	1.1	2
Hydrolyzable carbohy- drate.....	1.515	1.515	0.0	1.380	1.650	16.4	2.895	3.165	8.5	2
Reducing car- bohydrate..	0.5360	0.4640	13.4	0.4800	0.5360	10.4	1.016	1.000	1.6	2
Dry weight...	8.980	9.310	3.5	8.000	9.550	16.2	8.490	9.430	10.0	2

NALL (4) and determinations were made according to the usual methods (20). All samples were made in duplicate and checks were run on each sample. All differences greater than 2 per cent are significant except for ammonium, nitrate, and amide nitrogen. Here the variations between the several extractions differ by 15 per cent. The difficulties involved in sampling and extracting such small aliquots make the variability of results high.

Discussion

Cosmos is affected so strongly by a short daily light period that it will flower and set seed after being exposed to only ten 7½-hour

days. When flower primordia are initiated by a fewer number of short days the tendency to flower has not always been so definitely established that it may not be reversed by subsequent treatment with long days, producing "vegetative flowers." The short day treatment obviously has some marked effects on the chemical processes associated with flowering. The chemical changes accompanying the anatomical change agree closely with other observations concerning flowering and reproduction, especially in regard to the carbohydrate-nitrogen relation (16).

The composition of the whole plant does not change markedly, except that there is a decrease in dry weight of 10 per cent during the ten short days (17). This can be accounted for mainly by the decrease in polysaccharides of 8.5 per cent and a slight decrease in protein. As the amount of materials manufactured during the shortened days is markedly less, it seems important to consider the states of the metabolized materials as they are related to the daily light period. Under the shortened light exposure there is a marked tendency toward the concentration of soluble carbohydrate and complex protein in the stem tip at the expense of the polysaccharides and proteins in the base of the stem. There are differences in asparagine and ammonia and undoubtedly in many other cellular constituents, but a more complete study of the composition of the proteins is needed in order to give them meaning. The important point is that it requires approximately seven short days for the stem tip to acquire a definite and marked accumulation of reducing sugars, and a change in the state of the proteins toward a much more complex and difficultly soluble form. Any treatment involving fewer short days than is necessary to bring about this change is without effect in inducing flowering, but a few added days of treatment, once the accumulation has begun, giving time enough for the accentuation of the changes, insured 100 per cent conversion of the vegetative primordia to the flowering condition.

The initiation of a flower primordium is associated with a definite metabolic state of the stem tip. The changes in the metabolism of the vegetative plant which result in flowering due to photoperiodic treatment include a probable proteolysis of the proteinaceous material and the hydrolysis of polysaccharides in the base of the stem

and their removal to the stem tip. The photoperiod influences (1) the amount of material which the plant is able to conserve, (2) the use to which it will be put, and (3), the quality of the proteins and carbohydrates in the various regions.

The amount and kind of carbohydrate and protein as determined in these experiments, and the diurnal fluctuation in the amount of glutathione, seem to be factors intimately connected with the photoperiodic treatment which has a definite correlation with the morphological change of the primordium from vegetative to reproductive. Any conclusions as to the meaning of the fluctuation of glutathione content with the change in the daily light period cannot be made until more is known concerning the function of glutathione. In the light of certain recent findings by HAMMETT (14), it might seem that glutathione is a factor which modifies the metabolism of proteins. At any rate it seems evident that both carbohydrates and proteins are influenced by the photoperiod, but whether it is directly or indirectly, through the action of some intermediate compound, has not been determined.

Summary

1. The plants of *Cosmos sulphureus* Cav. var. Klondike reacting most readily to short day treatment required seven short days for the initiation of a flower primordium. The slowest plants required ten days. When the absolute minimum treatment necessary to induce flowering was given, followed by long days, there was a marked tendency toward the production of interphases between normal flowers and vegetative shoots.

2. The change of the primordium from foliar to floral was accompanied by a marked accumulation of carbohydrate and protein in the stem tip. There is a probable proteolysis and a decrease in protein in the base of the stem at the time of the conversion of the primordium to the flowering state. There is also hydrolysis of carbohydrates in the base of the stem and their removal to the stem tip at the same time.

3. The shortened light period caused a decrease of 1 per cent per day of the total materials stored.

4. There was a diurnal fluctuation of glutathione in the stem tip during the first seven days of short day treatment, but from the

time of the actual anatomical shift of the primordium from vegetative to flowering the total glutathione content remained permanently higher.

5. Asparagine was somewhat higher and ammonium somewhat lower in the stem tip at the time of flower bud formation.

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LITERATURE CITED

1. ADAMS, J., The effect on certain plants of altering the daily period of light. *Ann. Bot.* **37**:75-94. 1923.
2. ———, Duration of light and growth. *Ann. Bot.* **38**:509-523. 1924.
3. BENSLEY, R. R., and GERSH, I., Studies on cell structure by the freezing-drying method. *Anat. Rec.* **57**:228. 1925.
4. CHIBNALL, A. C., Investigations on the nitrogenous metabolism of the higher plants. Part III. The effect of low-temperature drying on the distribution of nitrogen in the leaves of the runner bean. *Biochem. Jour.* **16**:599-611. 1922.
5. ———, Investigations on the nitrogenous metabolism of the higher plants. Part VI. The rôle of asparagine in the metabolism of the mature plant. *Biochem. Jour.* **18**:395-405. 1924.
6. ECKERSON, SOPHIA H., Conditions affecting nitrate reduction by plants. *Contr. Boyce Thompson Inst.* **4**:119-130. 1932.
7. FINK, D. E., A micro method for estimating the relative distribution of glutathione in insects. *Science* **65**:143-145. 1927.
8. GARNER, W. W., and ALLARD, H. A., Effect of the relative length of day and night and other factors of the environment on growth and reproduction in plants. *Jour. Agr. Res.* **18**:553-606. 1920.
9. ———, Further studies in photoperiodism, the response of the plant to relative length of day and night. *Jour. Agr. Res.* **23**:871-920. 1923.
10. ———, Localization of the response in plants to relative length of day and night. *Jour. Agr. Res.* **31**:555-556. 1925.
11. GARNER, W. W., BACON, C. W., and ALLARD, H. A., Photoperiodism in relation to hydrogen-ion concentration of the cell sap and the carbohydrate content of the plant. *Jour. Agr. Res.* **27**:119-156. 1924.
12. GILBERT, B. E., The response of certain photoperiodic plants to differing temperature and humidity conditions. *Ann. Bot.* **40**:315-320. 1926.
13. ———, Interrelation of relative day length and temperature. *BOT. GAZ.* **81**:1-24. 1926.
14. HAMMETT, F. S., A possible explanation of the function of glutathione in developmental growth. *Science* **79**:457. 1934.

15. KNOTT, J. E., Further localization of the response in plant tissue to relative length of day and night. *Proc. Amer. Soc. Hort. Sci.* 23:67-70. 1926.
16. KRAUS, E. J., and KRAYBILL, H. R., Vegetation and reproduction with special reference to the tomato. *Oregon Agr. Exp. Sta. Bull.* 149. 1918.
17. LUBIMENKO, V. N., and SZEGLOVA, O. A., L'adaption photopériodique des plants. *Rev. Gén. Bot.* 40:675-689. 1928.
18. MURNEEK, A. E., Relation of carotinoid pigments to sexual reproduction in plants. *Science* 79:528. 1934.
19. NIGHTINGALE, G. T., The chemical composition of plants in relation to photoperiodic changes. *Univ. Wisconsin Agr. Exp. Sta. Bull.* 74. 1927.
20. NIGHTINGALE, G. T., SCHERMERHORN, L. G., and ROBBINS, W. R., Some effects of potassium deficiency on the histological structure and nitrogenous and carbohydrate constituents of plants. *New Jersey Agr. Exp. Sta. Bull.* 499. 1930.
21. SAKAGUCHI, S., Über eine neue Farbenreaktion von Protein und Arginine. *Jour. Biochem. Tokyo* 5:25-31. 1925.

